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Townsend and Townsend and Crew LLP

By: _____

PATENT
Attorney Docket No.: 17634-000512US
Client Reference N .: E-142-96/6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Brian R. Murphy, et al.

Application No.: 09/444,067

Filed: November 19, 1999

For: PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCES

Examiner: B. Brumback

Art Unit: 1642

INFORMAL COMMUNICATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Examiner Brumback:

Further to our telephone interview of October 2, 2000, please consider the following comments and enclosed materials pertaining to the Restriction Requirement mailed June 29, 2000 in the above-identified ('067) application. These comments and materials also pertain to a separate Restriction Requirement mailed June 7, 2000 in a related RSV application assigned to you (Serial No. 09/291,894 ('894)), and to a second related case only recently assigned to you and yet to be reviewed for possible restriction (Serial No. 09/444,221 ('221)). Applicants respectfully ask that you consider these comments and materials collectively to assist them in formulating responses to the outstanding Restriction Requirements in the first two related applications, and to evaluate any related issues in the third related application.

To facilitate this review, Applicants have attached a lineage map including restriction practice details for the '067 and '894 applications and their common parent application Serial No. 08/892,403 ('403) (now issued as U.S. Patent No. 5,993,824 ('824)). Also attached are copies of the pending claims in the '067, '894, and '221 applications, and the issued claims in the '824 patent. Considering the informal nature of this communication, it is respectfully urged that these materials, inclusive of this communication, be reserved for the Examiner's informal use only and excluded from entry in all official file wrappers.

As we discussed during the October 2nd phone interview, Applicants wish to work together with your office to prosecute the subject matter of the '067, '894, and '221 applications in an efficient, consistent manner. However, the Restriction Requirements in the first two cases alone proscribe a total of 33 separate groups proposed to constitute distinct inventions. It is noted that these applications are divisional ('067) and CIP ('894) applications, respectively, of the above-referenced, '403 common parent application. In the parent case, a Restriction Requirement was presented on December 10, 1997 which set forth only 9 groups proposed as distinct inventions. One of these groups was elected and prosecuted to become the issued '824 patent. Of the other groups specified in the parental Restriction Requirement, group II (relating to gene deletion or ablation mutants) was generally elected as the foundational subject matter of claims in '067 application, group III (chimeric RSV incorporating a heterologous gene or genome segment) for the '894 application, and groups V, VI and VIII (RSV with modification to a cis-acting regulatory sequence, including GS and GE signal sequences) for the '221 application. At the same time, Applicants recognize that the claims of the '067, '894, and '221 applications did not precisely follow the parental Restriction, but were restructured in part to consolidate subject matter from different proposed groups set forth therein (including polynucleotides, vaccines and methods relating to the claimed recombinant viruses). In addition, it is further recognized that the '894 is a CIP application which presented additional claimed embodiments related to the original invention.

Without addressing the specific merits of the pending Restriction Requirements, Applicants respectfully request that the proposed groupings be reconsidered in an effort to avert the high costs, inefficiency, and potentially inconsistent examination that would attend separate prosecution of 33 applications for the subject matter presented in the '067 and '894 applications. In this regard, we believe that many of the restricted groupings relate to species that are examinable together. In particular, it is submitted that certain dependent, and/or "combinatorial", aspects of the inventions are sufficiently related so as not to create an undue burden on the Office to examine them coordinately. At the same time, the high costs, inefficiency and potential inconsistency of examining this subject matter separately is believed to impose an undue burden on Applicants.

To explain these considerations by way of example, the '894 application is broadly directed to chimeric recombinant RSV incorporating a heterologous gene or genome segment within a recombinant RSV genome or antigenome. Two types of chimeric RSV are disclosed, chimeric human RSV (e.g., RSV incorporating an antigenic determinant of human RSV B in a human RSV A background) and human/non-human RSV (e.g., bovine-human RSV chimeras). Within each of these groups Applicants have provided a range of species which are united by their shared chimeric genome, but which have separate, dependent or combinatorial features. For example, human A/B chimeric RSV may incorporate a different heterologous gene or genome segment (e.g., of NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2), L, F or G) within the background genome. In yet more detailed aspects, a particular genome segment (e.g., a glycoprotein ectodomain) is exchanged or introduced to form the chimeric genome. These dependent aspects are believed to be at least sufficiently closely related such that their common examination would not impose an undue burden on the Office.

So too are the important "combinatorial" aspects of Applicants' recombinant RSV. In this regard, Applicants' invention depends in certain embodiments upon using a combination of features from a detailed "menu" of features to provide an effective, live-attenuated vaccine candidate. Returning to the example of a human RSV A/B chimera, it will often be desired to combine this basic chimeric construction with one or more attenuating mutations or other nucleotide changes that specify a desired phenotype. In this regard, Applicants have clearly shown how to introduce temperature-sensitive and other types of attenuating point mutations (see, e.g., groups IV-VI), as well as gene deletions (e.g., group VII) and other changes (e.g., group VIII), into chimeric human RSV. These combinatorial-designed viruses cannot be dissected away and separately prosecuted without undermining important purposes and advantages of the invention. For this reason, the Restriction Requirements in the above applications are believed to impose an undue burden on Applicants. To hopefully lessen this burden and ensure coordinated, efficient prosecution of the respective applications, further discussion with the Examiner to obtain official direction and guidance on these issues earnestly solicited.

Another possible avenue for consolidating examination of the '067 and '894 applications may be to examine together the subject matter embodied, for example, by recombinant, chimeric viruses; isolated polynucleotides encoding the recombinant, chimeric viral genome; immunogenic compositions comprising the recombinant viruses; methods for immunization involving administration of the recombinant viruses; and methods for producing the recombinant viruses. These related aspects of the invention are presently restricted in each of the subject applications. However, it is noted that the issued '824 patent from the '403 parent application includes claims that span a similar range of related subject matter. These different aspects are also believed to be at least sufficiently closely related such that their common examination would not impose an undue burden on the Office.

As a final note, we also discussed during our teleconference on October 2nd the restriction practice in a comparable case directed to recombinant parainfluenza viruses (PIVs), also out of the Murphy/Collins lab. Briefly, in Serial Number 09/083,793 ('793), the Examiner imposed a multi-group Restriction Requirement against claims that were generally of similar scope and diversity as those in the '403 parent RSV case. After Applicants argued that no "undue burden" would be imposed upon the Office to examine the claims together, the Examiner withdrew the Restriction Requirement, but imposed a 20-way species election requirement. After a reply submission by Applicants, arguing that a collective species examination would not impose an undue burden on the Office, this species election requirement was also withdrawn. In particular, the Examiner stated in an Office Action dated December 17, 1999 (Paper No. 10) as follows: "After search of the elected species, it was determined that search of the full scope of the invention would not be unduly burdensome, despite the large number of claims and species recited in the claims. Therefore the requirement for election of species is withdrawn." A copy of the pending claims in the '793 application is attached for your review and consideration.

In summary, Applicants' respectfully request that the Restriction Requirements in the '067 and '894 applications be reconsidered—with the objective of defining a more consolidated and coordinated approach for prosecuting the subject matter of these applications.

Toward this end, Applicants hope that the foregoing comments and enclosed materials will help clarify your position on these issues, to share with Applicants' representative in a proposed telephone interview some time during the next week if possible. This will assist Applicants' representative in preparing responses to the pending Restriction Requirements, which are now due on an urgent basis.

The Examiner is kindly invited to review the foregoing comments and materials and thereafter telephone the undersigned at 206-467-9600 if a timely interview can be scheduled to address the issues presented above.

Respectfully submitted,

Dated: October 19, 2000

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SE 5005275 v1

National Institutes of Health
RSV Portfolio

17634-000510US
NIH E-142-96/3
Application No. 08/892,403
Filed: 07/15/97
PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCES
Applicants: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng
U.S. Patent No. 5,993,824 Issued: 11/30/99
Restrict. Req. 12/10/97 Examiner: B. Brumbeck

Group I: Claims 2-10 drawn to recombinant RSV w/TS amino acid substitutions and methods of making recombinant RSV, and to claims 1, 47-51 and 61 as they read on the selected claims

Group II: Claims 13, 14, 27, 28, 52, and 53 drawn to recombinant RSV w/modified SH gene and to 1, 11, 12, 26 and 47-51 as they read on the selected claims

Group III: Claims 20, 31-34, 54, 56-58, and 62, drawn to recombinant RSV w/ heterologous RSV genes, and to claims 1, 11, 47-51, and 61 as they read on the selected claims

Group IV: Claims 21, 35, and 59, drawn to recombinant RSV w/ genome modifications to encode non-RSV proteins, and to claims 1, 26, and 47-51 as they read on the selected claims

Group V: Claim 19, drawn to recombinant RSV w/ a nucleotide modification in a translational start codon, and to claims 1 and 11 as they read on the selected claim

Group VI: Claims 18 and 38, drawn to recombinant RSV w/ a nucleotide modification in a termination codon or a GS or GE transcription signal, and to claims 1 and 11 as they read on selected claims

Group VII: Claims 22-25, 36, 37, and 60, drawn to recombinant RSV w/ a parainfluenza (PIV) gene and to claims 1, 11, 30, 47-51, 54, and 55 as they read on the selected claims

Group VIII: Claims 15-17, 29, 30, and 54 drawn to recombinant RSV w/ a nucleotide modification to a cis-acting regulatory sequence and to claims 1, 11, 30, 47-51, and 55 as they read on the selected claims

Group IX: Claims 39-46, drawn to a vaccine and methods of stimulating the immune system and to claims 26 and 38 to the extent that they read on the selected claims

Amndmt 2/10/98: Elected group I, claims 2-10
Amndmt 11/9/98: added claims 63 and 64, amended claims
Amndmt 4/27/99: amended claims only
Notice of Allowability 05/21/99
Allowed claims 1-10, 47-50, 61, 63 and 64 (renumbered as 1-17)

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National Institutes of Health
RSV Portfolio

CIP
15280P-001000US
NIH E-160-98/0
(formerly 17634-5-2)
Application No. 09/291,894
Filed: 04/13/99
PRODUCTION OF ATTENUATED CHIMERIC RSV VACCINES FROM CLONED NUCLEOTIDE SEQUENCES
Inventors: Collins, Murphy, Whitehead
Examiner: B. Brumback
Restrict. Req. 06/07/00:

Group I: Claims 1-3, 6-10, 21, 35, and 46-51 drawn to chimeric human RSV comprising RSV A combined with human RSV B

Group II: Claims 1, 4, 5, and 11, drawn to chimeric RSV wherein the heterologous gene is selected from NS1, NS2, N, P, M, SH, M1(ORF1), M2(ORF2), L, F, and G

Group III: Claims 1, 12, 13, 18-20, and 22, drawn to chimeric RSV further modified by attenuating mutations present within a panel of biologically derived mutant RSV strains

Group IV: Claims 1, 12, 14, 20, and 22, drawn to chimeric RSV incorporating temperature sensitive mutations

Group V: Claims 1, 12, 15, 20, and 22, drawn to chimeric RSV incorporating mutations from cold-passaged attenuated RSV

Group VI: Claims 1, 16, 17, 20, and 22, drawn to chimeric RSV comprising substituted F and o genes and further modified to incorporate attenuating point mutations

Group VII: Claims 1 and 22-24, drawn to chimeric RSV further comprising a nucleotide modification specifying a phenotypic change, wherein a SH, NS 1, NS2, M2ORF2, or G gene is deleted

Group VIII. Claims 1, 22, and 25-29, drawn to chimeric RSV further comprising a deletion, insertion, substitution or rearrangement of a cis-acting regulatory sequence

Group IX. Claims 1, 22, and 30-33, drawn to chimeric RSV incorporating a non-RSV gene

Group X. Claims 1 and 34, drawn to chimeric RSV comprising human RSV combined with bovine or murine RSV

Group XI. Claims 1 and 36, drawn to chimeric RSV which is a subviral particle

Group XII. Claims 1 and 37-45, drawn to methods of stimulating the immune system

Group XIII. Claims 52- 55 drawn to an isolated polynucleotide molecule comprising a chimeric RSV genome combined with a heterologous gene, wherein the heterologous gene encodes a RSV F, G, or SH glycoprotein

Group XIV. Claims 52, and 56-60, drawn to an isolated polynucleotide molecule comprising a chimeric RSV genome of subgroup A combined with a heterologous gene from subgroup B

Group XV. Claims 52 and 61-63, drawn to an isolated polynucleotide molecule comprising a chimeric RSV genome and further comprising a nucleotide modification specifying a phenotypic change

Group XVI. Claims 64 and 65, drawn to methods for producing an infectious attenuated chimeric RSV

PENDING

National Institutes of Health
RSV Portfolio

DIV
17634-000512US
NIH E-142-96/6
Application No. 09/444,067
Filed: 11/19/99
PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCES
Applicant: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng
Examiner: B. Brumback
Restrict Req. 6/29/00:

Group I: I. Claims 63-69, 120-122, 162, and 163, drawn to recombinant RSV with a gene deletion and to a method of making

Group II: Claims 63, 70-78, and 120-122, drawn to recombinant RSV wherein expression of a selected gene is reduced or ablated

Group III: Claims 63, 79-87, and 120-122, drawn to recombinant RSV wherein the position of one or more genes is altered relative to an RSV promotor

Group IV: Claims 63, 88-95, and 120-122, drawn to recombinant RSV with a modification modulating a change in phenotype or with attenuating mutations

Group V: Claims 63, 96-107, and 120-122, drawn to recombinant RSV with heterologous RSV genes

Group VI: Claims 63, 108-115, and 120-122, drawn to recombinant RSV with a nucleotide modification to a cis-acting regulatory sequence

Group VII: Claims 63, 116-118, and 120-122, drawn to recombinant RSV with a Ply gene

Group VIII Claims 63 and 119-122, drawn to recombinant RSV encoding a non-RSV molecule

Group IX: Claims 63 and 120-131, drawn to a vaccine and a method of stimulating the immune system

Group X: Claims 133-138, drawn to isolated RSV polynucleotides and expression vectors

Group XI: Claims 133 and 139-145, drawn to RSV polynucleotides wherein expression of a selected gene is reduced or ablated

Group XII: Claims 133 and 146, drawn to RSV polynucleotides wherein the position of one or more genes is altered relative to an RSV promotor

Group XIII: Claims 133 and 147-150, drawn to RSV polynucleotides with modifications modulating a change in phenotype or with attenuating mutations

Group XIV: Claims 133 and 151-155, drawn to RSV polynucleotides with heterologous RSV genes

Group XV: Claims 133 and 156-159, drawn to isolated RSV polynucleotides with a nucleotide modification to a cis-acting regulatory sequence

Group XVI: Claims 133 and 160, drawn to isolated RSV polynucleotides with a PIV gene

Group XVII: Claims 133 and 161, drawn to isolated RSV polynucleotides encoding a non-RSV molecule

PENDING

DIV
17634-000513US
NIH E-142-96/8
Application No. 09/444,221
Filed: 11/19/99
PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCES
Applicant: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng
Examiner: B. Brumbeck

PENDING

National Institutes f Health
RSV Portfolio
Claims

DIV
15280-000512US
NIH E-142-96/6
Application No. 09/444,067
Filed: 11/19/99
PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCESApplicant: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng

PENDING CLAIMS:

63. An isolated infectious recombinant respiratory syncytial virus (RSV) comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase elongation factor, wherein a modification is introduced within the genome or antigenome comprising a partial or complete gene deletion, a change in gene position, or one or more nucleotide change(s) that modulate expression of a selected gene.

64. The recombinant RSV of claim 63, wherein said gene is selected from an attachment (G) protein, fusion (F) protein, small hydrophobic (SH) protein, RNA binding protein (N), phosphoprotein (P), large polymerase protein (L), M2(ORF1) or M2(ORF2) product, matrix (M) protein, or a nonstructural protein NS1 or NS2.

65. The recombinant RSV of claim 63, wherein a RSV gene is deleted in whole or in part.

66. The recombinant RSV of claim 65, wherein a SH, NS1, NS2, or G gene is deleted in whole or in part.

67. The recombinant RSV of claim 66, wherein the SH gene is deleted.

68. The recombinant RSV of claim 66, wherein the NS1 gene is deleted.

69. The recombinant RSV of claim 66, wherein the NS2 gene is deleted.

70. The recombinant RSV of claim 63, wherein expression of a selected RSV gene is reduced or ablated by introduction of one or more translation termination codons. 71. The recombinant RSV of claim 70, wherein expression of a selected RSV gene is reduced or ablated by introduction of multiple translation termination codons.

72. The recombinant RSV of claim 71, wherein expression the RSV NS2 gene is reduced or ablated by introduction of multiple translation termination codons

73. The recombinant RSV of claim 63, wherein expression of a selected RSV gene is reduced or ablated by introduction of a frame shift mutation in the gene.

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Claims

DIV
15280-000512US
NIH E-142-96/6
Application No. 09/444,067
Filed: 11/19/99
PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCESApplicant: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng

74. The recombinant RSV of claim 63, wherein expression of a selected RSV gene is modulated by introduction, modification or ablation of a translational start site within the gene.

75. The recombinant RSV of claim 74, wherein a translational start site of the selected gene is modified or ablated to prevent efficient translation initiation at said start site.

76. The recombinant RSV of claim 74, wherein an internal translational start site of the selected gene is modified or ablated to prevent efficient translation initiation at said start site.

77. The recombinant RSV of claim 74, wherein an internal translational start site of the RSV G gene is ablated to prevent efficient translation initiation at said start site specifying expression of a secreted form of the G protein.

78. The recombinant RSV of claim 74, wherein a translational start site is introduced upstream of the selected gene or internally to enhance expression of the gene.

79. The recombinant RSV of claim 63, wherein a position of one or more gene(s) in the genome or antigenome is altered relative to a RSV promoter.

80. The recombinant RSV of claim 79, wherein a position of said one or more gene(s) is changed to a more promoter-proximal location specifying enhanced expression of the gene(s).

81. The recombinant RSV of claim 80, wherein said position of said one or more gene(s) is changed to a more promoter-proximal location by deletion of coding or non-coding sequences within the genome or antigenome upstream of said one or more gene(s).

82. The recombinant RSV of claim 81, wherein positions of multiple RSV gene(s) are changed to a more promoter-proximal location by deletion of a SH or NS2 gene or genome segment.

83. The recombinant RSV of claim 79, wherein a position of said one or more gene(s) is changed to a more promoter-distal location specifying reduced expression of the gene(s).

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84. The recombinant RSV of claim 81, wherein a coding or non-coding polynucleotide sequence selected from an autologous or heterologous RSV or non-RSV gene or gene segment is inserted in the genome or antigenome upstream of said one or more gene(s).

85. The recombinant RSV of claim 79, wherein positions of multiple genes in the genome or antigenome are altered by changing their relative gene order.

86. The recombinant RSV of claim 85, wherein the positions of multiple genes are altered by reciprocal positional substitution of said genes in the genome or antigenome.

87. The RSV of claim 86, wherein the NS2 gene is reciprocally substituted in position for the SH gene.

88. The recombinant RSV of claim 63, wherein said modification within the genome or antigenome comprising a partial or complete gene deletion, a change in gene position, or one or more nucleotide change(s) that modulate expression of a selected gene specifies a change in phenotype for the resultant recombinant virus selected from a change in growth characteristics in culture, small plaque size, attenuation in vivo, temperature-sensitivity, cold-adaptation, host range restriction, change in antigen expression, or a change in immunogenicity.

89. The recombinant RSV of claim 63, wherein the genome or antigenome is further modified to incorporate one or more attenuating mutation(s) present in one or more biologically derived mutant human RSV strain(s).

90. The recombinant RSV of claim 89, wherein the genome or antigenome is further modified to incorporate at least one and up to a full complement of attenuating mutations present within a panel of biologically derived mutant human RSV strains, said panel comprising cpts RSV 248 (ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts RSV 248/955 (ATCC VR 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009 (ATCC VR 2451), cpts RSV 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC VR 2542), and RSV B-1 cp-23 (ATCC VR 2579).

91. The recombinant RSV of claim 89, wherein the genome or antigenome is further modified to incorporate at least one and up to a full complement of attenuating mutations specifying an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or Thr523 in the RSV F gene, Cys319, Phe 521, Gln831, Met1169, Tyr1321 and/or His 1690 in the RSV polymerase gene L, and a nucleotide substitution in the gene-start sequence of gene M2.

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15280-000512US
NIH E-142-96/6
Application No. 09/444,067
Filed: 11/19/99
PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCESApplicant: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng

92. The recombinant RSV of claim 89, wherein the genome or antigenome is further modified to incorporate at least one mutation specifying a temperature-sensitive substitution at amino acid Phe521, Gln831, Met1169, or Tyr1321 in the RSV polymerase gene or a temperature- sensitive nucleotide substitution in the gene-start sequence of gene M2.

93. The recombinant RSV of claim 89, wherein the genome or antigenome incorporates at least two attenuating mutations.

94. The RSV of claim 1, having at least three attenuating mutations.

95. The recombinant RSV of claim 89, wherein the genome or antigenome includes at least one attenuating mutation stabilized by multiple nucleotide changes in a codon specifying the mutation.

96. The recombinant RSV of claim 63, wherein the genome or antigenome comprises a partial or complete human RSV genome or antigenome of one RSV subgroup or strain combined with a heterologous gene or gene segment from a different, human or non-human RSV subgroup or strain to form a chimeric genome or antigenome. 97. The recombinant RSV of claim 96, wherein the heterologous gene or gene segment is from a human RSV subgroup A, human RSV subgroup B, bovine RSV, or murine RSV.

98. The recombinant RSV of claim 96, wherein the heterologous gene or gene segment is selected from a RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2), L, F or G gene or gene segment.

99. The recombinant RSV of claim 96, wherein the chimeric genome or antigenome comprises a partial or complete human RSV A subgroup genome or antigenome combined with a heterologous gene or gene segment from a human RSV B subgroup virus.

100. The recombinant RSV of claim 99, wherein the heterologous gene or gene segment from human RSV B encodes a RSV F, G or SH glycoprotein or a cytoplasmic domain, transmembrane domain, ectodomain or immunogenic epitope thereof. 101.The recombinant RSV of claim 100, wherein one or more human RSV B subgroup glycoprotein genes F, G and SH or a cytoplasmic domain, transmembrane domain, ectodomain or immunogenic epitope thereof is substituted within a partial RSV A genome or antigenome.

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102. The recombinant RSV of claim 101, wherein both human RSV B subgroup glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein genes in the RSV A genome or antigenome.

103. The recombinant RSV of claim 96, wherein the chimeric genome or antigenome comprises a partial or complete human RSV B subgroup genome or antigenome combined with a heterologous gene or gene segment from a human RSV A subgroup virus.

104. The recombinant RSV of claim 63, wherein the chimeric genome or antigenome comprises a partial or complete RSV background genome or antigenome of a human or bovine RSV combined with a heterologous gene or genome segment of a different RSV to form a human-bovine chimeric RSV genome or antigenome.

105. The recombinant RSV of claim 104, wherein the heterologous gene or genome segment is substituted for a counterpart gene or genome segment in a partial RSV background genome or antigenome.

106. The recombinant RSV of claim 104, wherein the heterologous gene or genome segment is added adjacent to or within a noncoding region of the partial or complete RSV background genome or antigenome.

107. The recombinant RSV of claim 104, wherein the chimeric genome or antigenome comprises a partial or complete human RSV background genome or antigenome combined with a heterologous gene or genome segment from a bovine RSV.

108. The recombinant RSV of claim 63, wherein the genome or antigenome is further modified to incorporate a nucleotide deletion, insertion, substitution, rearrangement, or modification of a cis-acting regulatory sequence within the recombinant RSV genome or antigenome.

109. The recombinant RSV of claim 108, wherein the cis-acting regulatory sequence occurs within a 3' leader, 5' trailer or intergenic region of the RSV genome or antigenome.

110. The recombinant RSV of claim 108, wherein the cis-acting regulatory sequence is a gene-start (GS) signal, a (GE) signal, or a RSV promoter element.

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PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCESApplicant: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng

111. The recombinant RSV of claim 108, wherein the cis-acting regulatory sequence is a gene-start (GS) or gene-end (GE) signal which is modified, deleted, inserted or is replaced by a heterologous GS or GE signal in the genome or antigenome.

112. The recombinant RSV of claim 111, wherein a GE signal of the RSV NS1 or NS2 gene is replaced by a corresponding GE signal of the RSV N gene.

113. The recombinant RSV of claim 108, wherein the cis-acting regulatory sequence is replaced by a heterologous regulatory sequence.

114. The recombinant RSV of claim 113, wherein the heterologous regulatory sequence is a cis-acting regulatory sequence of a different RSV gene.

115. The recombinant RSV of claim 108, wherein a RSV promoter element is replaced by a heterologous promoter from a different RSV.

116. The recombinant RSV of claim 63, wherein the genome or antigenome incorporates a heterologous gene or genome segment from parainfluenza virus (PIV).

117. The recombinant RSV of claim 116, wherein the gene or genome segment encodes a PIV HN or F glycoprotein or immunogenic domain or epitope thereof.

118. The recombinant RSV of claim 116, wherein the genome segment encodes one or more immunogenic protein(s), protein domain(s) or epitope(s) HPIV1, HPIV2, and/or HPIV3.

119. The recombinant RSV of claim 63, wherein the genome or antigenome is further modified to encode a non-RSV molecule selected from a cytokine, a T-helper epitope, or a protein of a microbial pathogen capable of eliciting a protective immune response in a mammalian host.

120. The recombinant RSV of claim 63 which is a virus.

121. The recombinant RSV of claim 63 which is a subviral particle.

122. The recombinant RSV of claim 63, formulated in a dose of 10^3 to 10^6 PFU of attenuated virus.

National Institutes of Health
RSV Portfolio
Claims

DIV
15280-000512US
NIH E-142-96/6
Application No. 09/444,067
Filed: 11/19/99
PRODUCTION OF ATTENUATED RESPIRATORY SYNCYTIAL VIRUS VACCINES FROM CLONED NUCLEOTIDE SEQUENCESApplicant: Murphy, Collins, Whitehead, Bukreyev, Juhasz and Teng

123. A method for stimulating the immune system of an individual to induce protection against respiratory syncytial virus, which comprises administering to the individual an immunologically sufficient amount of the recombinant RSV of claim 63. 124. The method of claim 123, wherein the recombinant virus is administered in a dose of 10^3 to 10^6 PFU of the attenuated RSV.

125. The method of claim 123, wherein the recombinant virus is administered to the upper respiratory tract.

126. The method of claim 125, wherein the recombinant virus is administered by spray, droplet or aerosol.

127. The method of claim 123, wherein the recombinant virus is administered to an individual seronegative for antibodies to RSV or possessing transplacentally acquired maternal antibodies to RSV.

128. A vaccine to induce protection against RSV, which comprises an immunologically sufficient amount of the recombinant RSV of claim 63 in a physiologically acceptable carrier.

129. The vaccine of claim 128, formulated in a dose of 10^3 to 10^6 PFU of the attenuated RSV.

130. The vaccine of claim 128, formulated for administration to the upper respiratory tract by spray, droplet or aerosol.

131. The vaccine of claim 128, wherein the recombinant RSV elicits an immune response against human RSV A, human RSV B, or both.

132. An expression vector comprising an isolated polynucleotide molecule encoding a respiratory syncytial virus (RSV) genome or antigenome modified by a partial or complete gene deletion, a change in gene position, or one or more nucleotide change(s) that modulate expression of a selected gene.

133. An isolated polynucleotide molecule comprising a respiratory syncytial virus (RSV) genome or antigenome which is modified by a partial or complete gene deletion, a

change in gene position, or one or more nucleotide change(s) that modulate expression of a selected gene.

134. The isolated polynucleotide molecule of claim 133, wherein a RSV gene is deleted in whole or in part.

135. The isolated polynucleotide molecule of claim 134, wherein a SH, NS1, NS2, or G gene is deleted in whole or in part.

136. The isolated polynucleotide molecule of claim 135, wherein the SH gene is deleted.

137. The isolated polynucleotide molecule of claim 135, wherein the NS1 gene is deleted.

138. The isolated polynucleotide molecule of claim 135, wherein the NS2 gene is deleted.

139. The isolated polynucleotide molecule of claim 133, wherein expression of a selected RSV gene is reduced or ablated by introduction of one or more translation termination codons. 140. The isolated polynucleotide molecule of claim 133, wherein expression of a selected RSV gene is reduced or ablated by introduction of a frame shift mutation in the gene.

141. The isolated polynucleotide molecule of claim 133, wherein expression of a selected RSV gene is modulated by introduction, modification or ablation of a translational start site within the gene.

142. The isolated polynucleotide molecule of claim 141, wherein a translational start site of the selected gene is modified or ablated to prevent efficient translation initiation at said start site.

143. The isolated polynucleotide molecule of claim 141, wherein an internal translational start site of the selected gene is modified or ablated to prevent efficient translation initiation at said start site.

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144. The isolated polynucleotide molecule of claim 143, wherein an internal translational start site of the RSV G gene is ablated to prevent efficient translation initiation at said start site specifying expression of a secreted form of the G protein.

145. The isolated polynucleotide molecule of claim 141, wherein a translational start site is introduced upstream of the selected gene or internally to enhance expression of the gene.

146. The isolated polynucleotide molecule of claim 133, wherein a position of one or more gene(s) in the genome or antigenome is altered relative to a RSV promoter.

147. The isolated polynucleotide molecule of claim 133, wherein said modification within the genome or antigenome comprising a partial or complete gene deletion, a change in gene position, or one or more nucleotide change(s) that modulate expression of a selected gene specifies a change in phenotype for the resultant recombinant virus selected from: a change in growth characteristics in culture, small plaque size, attenuation in vivo, temperature-sensitivity, cold-adaptation, host range restriction, change in antigen expression, or a change in immunogenicity.

148. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome is further modified to incorporate one or more attenuating mutation(s) present in one or more biologically derived mutant human RSV strain(s).

149. The isolated polynucleotide molecule of claim 148, wherein the genome or antigenome is further modified to incorporate at least one and up to a full complement of attenuating mutations specifying an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or Thr523 in the RSV F gene, Cys319, Phe 521, Gln831, Met1169, Tyr1321 and/or His 1690 in the RSV polymerase gene L, and a nucleotide substitution in the gene-start sequence of gene M2.

150. The isolated polynucleotide molecule of claim 148, wherein the genome or antigenome incorporates at least two attenuating mutations.

151. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome comprises a partial or complete human RSV genome r antigenome of one RSV subgroup or strain combined with a heterologous gene or gene segment from a different, human or non-human RSV subgroup or strain to form a chimeric genome or antigenome. 152. The

isolated polynucleotide molecule of claim 151, wherein the heterologous gene or gene segment is from a human RSV subgroup A, human RSV subgroup B, bovine RSV, or murine RSV.

153. The isolated polynucleotide molecule of claim 152, wherein the chimeric genome or antigenome comprises a partial or complete human RSV A subgroup genome or antigenome combined with a heterologous gene or gene segment from a human RSV B subgroup virus.

154. The isolated polynucleotide molecule of claim 63, wherein the chimeric genome or antigenome comprises a partial or complete RSV background genome or antigenome of a human or bovine RSV combined with a heterologous gene or genome segment of a different RSV to form a human-bovine chimeric RSV genome or antigenome.

155. The isolated polynucleotide molecule of claim 154, wherein the chimeric genome or antigenome comprises a partial or complete human RSV background genome or antigenome combined with a heterologous gene or genome segment from a bovine RSV.

156. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome is further modified to incorporate a nucleotide deletion, insertion, substitution, rearrangement, or modification of a cis-acting regulatory sequence within the recombinant RSV genome or antigenome.

157. The isolated polynucleotide molecule of claim 156, wherein the cis-acting regulatory sequence is a gene-start (GS) signal, a gene-end (GE) signal, or a RSV promoter element.

158. The isolated polynucleotide molecule of claim 157, wherein the cis-acting regulatory sequence is a gene-start (GS) or gene-end (GE) signal which is modified, deleted, inserted or is replaced by a heterologous GS or GE signal in the genome or antigenome.

159. The isolated polynucleotide molecule of claim 158, wherein a GE signal of the RSV NS1 or NS2 gene is replaced by a corresponding GE signal of the RSV N gene.

160. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome incorporates a heterologous gene or genome segment from parainfluenza virus (PIV).

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161. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome is further modified to encode a non-RSV molecule selected from a cytokine, a T-helper epitope, or a protein of a microbial pathogen capable of eliciting a protective immune response in a mammalian host.

162. A method for producing a recombinant respiratory syncytial virus (RSV) from one or more isolated polynucleotide molecules encoding said RSV, comprising:

expressing in a cell or cell-free lysate an expression vector comprising an isolated polynucleotide comprising a recombinant RSV genome or antigenome which is modified by a partial or complete gene deletion, a change in gene position, or one or more nucleotide change(s) that modulate expression of a selected gene.

163. The method of claim 162, wherein the recombinant RSV genome or antigenome and the N, P, L and RNA polymerase elongation factor proteins are expressed by two or more different expression vectors.